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Evolutionary reproductive morphology of amphibians: an overview

Susanne Kühnel[1], Sandy Reinhard[1] & Alexander Kupfer[1,2*]
[1] Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum, Friedrich-Schiller-Universität Jena, Erbertstr. 1, D-07743 Jena, Germany
[2] Biologie und Didaktik, Universität Siegen, Adolf-Reichwein-Str. 2, D-57068 Siegen
* Correspondence; E-mail: alexander.kupfer@uni-jena.de

Abstract. Reproduction is a crucial trait in the life history of any organism, and vertebrates, whether aquatic or terrestrial, have evolved an extraordinary diversity of reproductive strategies and morphologies. Among tetrapods, the diversity of reproductive modes is exceptionally high in amphibians, who also show multiple trends towards terrestrialisation and internal fertilisation. Herein we give a brief overview of the diversity of amphibian reproductive morphology, with a special emphasis on the cloaca, for all three major lineages, i.e., anurans, urodeles and caecilians.

Key words. Reproduction, genital morphology, Amphibia.

INTRODUCTION

Reproduction is a crucial trait in the life history of any organism and scientists have been intrigued and challenged by this event, and the structures associated with it, ever since the days of Darwin (1871). Both aquatic and terrestrial vertebrates have evolved an extraordinary diversity of reproductive strategies and morphologies, including varieties of oviparity and viviparity (Meisenheimer 1921; Lombardi 1998). Among tetrapods, the diversity of reproductive modes is exceptionally high in amphibians. In this group, we also see multiple trends towards terrestrialisation and internal fertilisation (e.g. Haddad & Prado 2005). Reproductive modes such as viviparity have evolved independently in all three lineages of modern amphibians (e.g. Noble 1927; Wake & Dickie 1998; Wells 2007). Herein we interpret a reproductive mode as a combination of several reproductive traits, including oviposition site, clutch characteristics, stage and size of hatchling, and type of parental care (sensu Salthe 1969).

Internal fertilization is a precondition for viviparity (Wourms & Lombardi 1992; Böhme & Ziegler 2008). It is associated with different strategies of sperm transfer, which have evolved within all three amphibian orders, ranging from cloacal apposition in anurans to a true copula via a male intromittent organ in caecilians (Sever et al. 2001; Kupfer et al. 2006). Like most other tetrapods, amphibians have a cloaca, a chamber that receives products from the kidneys, the intestine and the gonads, and opens to the outside through a cloacal opening or vent (Kardong 2006).

Below we review the diversity of amphibian cloacal morphologies involved in ensuring a secure direct sperm transfer and internal fertilization among anurans, urodeles and caecilians.

REPRODUCTIVE MORPHOLOGY OF AMPHIBIANS

Anura

The majority of anurans, currently including almost 6000 species (AmphibiaWeb 2010), practice external fertilization, and thus have no special male cloacal arrangements facilitating direct sperm transfer (recently summarised by Wells 2007). During copulation, males grasp females firmly with their forearms (termed amplexus). In most cases, sperm is directly released on the eggs protruding from the female cloaca, but in some cases fertilisation takes place without amplexus (e.g. Crump 1974; Kunte 2004). Internal fertilisation is rare among anurans, and mostly connected to viviparity or other complex parental care mechanisms (e.g. Wake 1993; Beck 1998).

Exceptionally, the phylogenetically basal tailed frogs *Ascaphus truei* and *A. montanus* are the only anurans known to have evolved a true intromittent organ in males (see Figs 1A–B). During courtship they practise a combination of amplexus and copulation called "copulexus" (see Sever et al. 2001; Stephenson & Verrell 2003). The so-called

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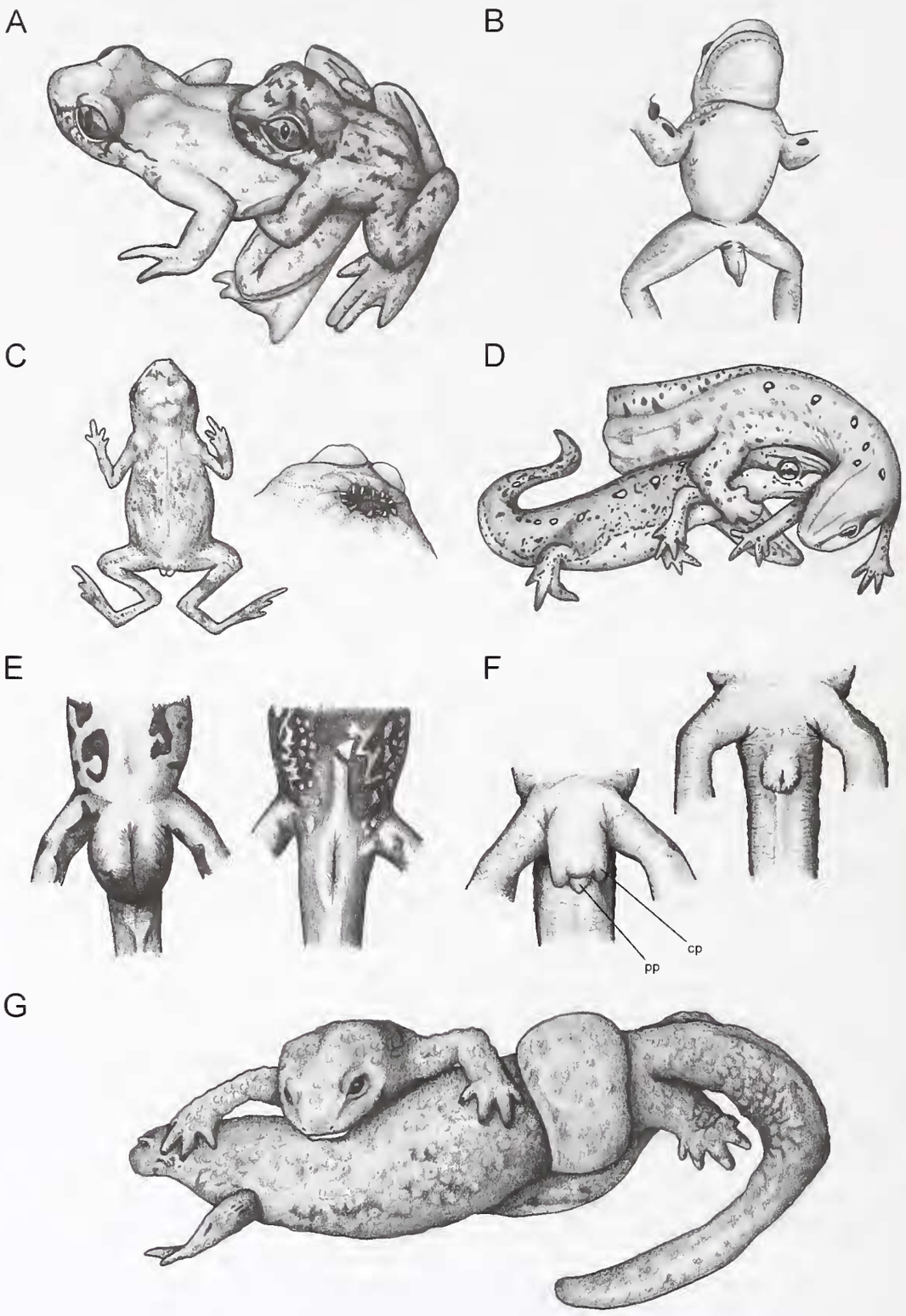

Fig. 1. Reproductive morphology of anurans and salamanders. (A) Inguinal amplexus ("copulexus") of *Ascaphus truei*. (B) male *Ascaphus truei*. The "tail", a cloacal extension, can be inserted into the cloaca of the female during amplexus, ventrolateral view. (C) male *Mertensophryne micranotis* (Anura: Bufonidae), left, dorsal view and its cloaca, right, caudal view (after Grandison 1980). (D) cephalic amplexus of *Notophthalmus viridescens* (Urodela: Salamandridae). The male grasps the females's neck whilst fanning pheromones towards her nostrils. (E) cloacal region of lentic breeding *Cynops pyrrhogaster* (Urodela: Salamandridae). The male's cloaca (left) is heavily swollen compared to that of the female (right). (F) cloacal region of lotic breeding *Euproctus montanus* (Urodela: Salamandridae, after Brizzi et al. 1995). Males (left) possess a cloacal protuberance (cp) which bears a protusible pseudopenis (pp), whereas the female cloaca is slightly conical shaped and its opening is located ventrally (right). (G) amplecting pair of *Calotriton arnoldi* (Urodela: Salamandridae). The male grasps the female's trunk with his tail.

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"tail" resembles the posteriorly extended cloaca, proximately attended by Nobelian rods and strengthened by vascularized tissue that is engorged with blood during copulation. This gives the ventral cloacal surface a pinkish colour (Noble & Putnam 1931; Duellman & Trueb 1994). To insert the posterior pointing "tail" into the female vent, the male first flexes his pelvis at a right angle to the vertebral column. Contraction of the paired Musculi compressores cloacae (Duellman & Trueb 1994) bend the intromittent organ ventrally, with the male vent pointing anteriorly (Slater 1931). Keratinised spines are present within the male cloacal orifice, but whether they function to enhance the attachment of the male to the female remains unclear (Noble & Putnam 1931; Metter 1964).

Additionally, internal fertilisation including an amplexus and cloacal apposition occurs in a few anurans, such as several species of viviparous African dwarf toads *Nectophrynoides* (Wake 1980; Wake & Dickie 1998) and *Nimbaphrynoides* (Sandberger et al. 2010), and in two species of Caribbean *Eleutherodactylus*, the viviparous *E. jasperi* (Dewry & Kirkland 1976; Wake 1978) and the oviparous-direct developing *E. coqui* (Townsend et al. 1981). Mating has only been observed in couples of *E. coqui* in a special amplectic position called reverse hind leg clasp, that is initiated by the female (Townsend & Stewart 1986). Males do not clasp, and the female rests her hind legs on top of the male´s legs. This behaviour might be correlated with terrestrial reproduction and internal fertilization. It is also thought to be present in the viviparous *E. jasperi* but has not yet been observed (Wake 1978). Within the African Bufonidae, all species of *Nectophrynoides* (and also *Altiphrynoides malcolmi* and *Nimbaphrynoides occidentalis*, former members of *Nectophrynoides,* see Frost et al. 2006) practice internal fertilization. *Altiphrynoides* and *Nimbaphrynoides* both show a dimorphism of the male and female vent, and an inguinal amplexus in a unique belly-to-belly position has been reported as well (Grandison 1978).

As in the internally fertilising *Ascaphus* ssp., males of the East African toad *Mertensophryne micranotis* (Bufonidae) exhibit modifications of the cloacal region (Duellman & Trueb 1994). They have small conical spines around the rim of the vent and at the entrance to the cloacal tube restricted to the ridges of the puckered vent (Grandison 1980, see also Fig 1C). Males and females keep a very tight cloacal contact during mating. Although the cloacal spines play a role to ensure a close apposition of the vents, to secure internal fertilisation, there is no evidence for a direct interlocking mechanism in the furrows of the female vent (Grandison & Ashe 1983).

Another potential record of internal fertilisation is provided for the neotropical Pumpkin Toadlet *Brachycephalus ephippium* (Pombal et al. 1994). During mating, males shift from an inguinal to an axillary amplexus to optimally allow positioning of the vents, and thus maximize fertilization of the relatively large eggs (5.1 to 5.3 mm). A further record of viviparity in fanged frogs (*Limnonectes* spec.) from Sulawesi probably also involves internal fertilisation(Emerson 2001).

It can be hypothesised that (1) many terrestrially breeding species with large direct-developing clutches are internal fertilizers and (2) if additional viviparous species are encountered they will also show internal fertilisation. Thus, internal fertilisation and viviparity in anurans might be more widespread than currently recognized (see also Wake 1978).

Data on the reproductive biology, including the mating behaviour, of many species is still lacking (Duellman & Trueb, 1994; Wells 2007). Life history data from around 23 % of the currently known species is missing, as listed in the data deficient category of the IUCN (Stuart et al. 2008).

Urodela

The majority of the 590 species of urodeles exhibit internal fertilization, whilst only males of the basal families Hynobiidae, Cryptobranchidae, and presumably Sirenidae, fertilise eggs externally (summarised in Duellmann & Trueb 1994; Wells 2007). The complex and elaborate courtship behavior of most salamanders includes the deposition of a spermatophore by the male, which is subsequently received by the female. A true intromittent organ in salamanders is lacking, although direct sperm transfer can be found in one species – the Corsican brook newt *Euproctus montanus*, a lotic breeding endemic of the island of Corsica. The cloaca of the male brook newt resembles a conical protuberance (Fig 1F). The cloacal chamber hosts a "pseudopenis", a broad conspicuous papilla, which can be evaginated during mating (Brizzi et al. 1995; Carranza & Amat 2005). The male grasps the female during amplexus, holding her tail with his jaws and wrapping his tail around her trunk, whilst his backward projecting cloaca is positioned close to that of the female. A deep groove along the ventral surface of the pseudopenis, which is aligned with the cloacal tube, guarantees a guided, unidirectional flux of cloacal products. Thus, sperm mixed with secretory products is transferred directly into the female's cloaca. The Salamandroidea that practice internal fertilization possess a distinct set of male cloacal glands necessary for spermatophore production (Sever 2002). The glands are hormonally controlled and hypertrophied during the breeding season, often causing a sexual dimorphism in cloacal shape. However, in *Euproc-*

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tus montanus, cloacal glands are reduced or partly lacking (Brizzi et al. 1995; Sever 2002). Males of six salamandrid genera possess a so-called "pseudopenis", a projection of the dorsal roof which nearly fills the entire anterior chamber of the cloaca. It is involved in shaping and expulsion of the spermatophore (Halliday 1998), but cannot be everted as in the Corsican brook newt (Brizzi et al. 1995; Carranza & Amat 2005).

Sexes of most species, regardless of the mode of fertilisation, show a sexual dimorphism in cloacal shape (Figs 1E–F). Usually, the male cloaca appears larger and more swollen than the female one. This is caused by the activity of the glands mentioned above (see also Sever 2002). Species that breed in the water and show elaborate courtship dances or walks, such as some members of the family Salamandridae, produce courtship pheromones, which are fanned towards the female using the tail. Salamanders that mate terrestrially also use courtship pheromones secreted from specialised glands to attract females. Pheromone-producing cloacal glands are therefore highly influenced by sexual selection (e.g. Sever 2002; Houck et al. 2008). Usually, female cloacae are less prominent, but they may also possess up to three types of cloacal glands in Salamandroidea, mainly accounting for sperm storage (spermathecae), a unique feature among vertebrates (Sever 1994). Females may retain and mix viable spermatozoa from multiple matings in the spermathecae for longer periods (e.g. Steinfartz et al. 2006). Female *Eurycea* fertilise eggs from stored sperm up to eight months after insemination, female *Notophthalmus viridescens* effectively store sperm for up to six months, and female *Salamandra salamandra* are reported to store sperm for up to two years (Sever et al. 1996; Stebbins & Cohen 1997; Sever & Brizzi 1998).

Additionally, the shape of female cloacae can be adapted to a specific substrate for oviposition and type of water body. Females of stream-breeding species, such as *Calotriton asper*, sometimes have a conically shaped cloaca for egg deposition and safe attachment between stones and in crevices (e.g. Carranza & Amat 2005).

Lotic breeders such as *Calotriton* ssp. often engage in an amplexus directly transferring the spermatophore into the female cloaca (Fig 1G). It ensures direct and rapid spermatophore uptake, and thus reduces energy wasting, which can hardly be avoided during aquatic breeding where the male and the female often have no physical contact. Breeding patterns including an amplexus are common in salamandrids. Multiple ways of female capture are known, such as the cephalic capture of *Notophthalmus* ssp. (see Fig 1D), the dorsal capture of *Taricha*, or the ventral capture performed by fire salamanders (Stebbins & Cohen 1997). The mating amplexus may last up to several hours, depending on the species. Salamanders of the family Ambystomatidae mate in the water, and the males guide females to spermatophore-uptake using a "tail-nudging-walk", except in *Ambystoma gracile*, *A. laterale*, *A. jeffersonarium* and *A. macrodactylum*, which capture females in an amplexus (Duellmann & Trueb 1994; Verrell & Krenz 1998). In contrast, some plethodontids perform a unique "tail-straddling-walk" behaviour (e.g. Arnold 1977).

Gymnophiona

In contrast to all salamanders (with the exception of *Euproctus montanus*) and frogs (with the exception of *Ascaphus* ssp.), the male caecilian cloaca is evertible through the vent and operates as an intromittent organ or phallus, a unique structure among tetrapods (Tonutti 1931, see also Fig 2A). Presumably all ca. 190 caecilian species (oviparous and viviparous) practice internal fertilisation with the help of the phallodeum (Tonutti 1931; Wake 1972; Gower & Wilkinson 2002), which is inserted into the female vent during copulation (e.g. Kupfer et al. 2006a).

The caecilian vent is simply surrounded by several folds, which are variably arranged among the groups and display sexual dimorphism in some species, such as members of the Typhlonectidae (e.g. Taylor 1968; Kupfer 2007). In contrast, the cloaca is highly complex and diverse. The male caecilian cloaca is an elongated tube divided into two distinct chambers. The cranial urodeum is rather simply built, bearing longitudinal ridges, and connects to the intestine and the urogenital ducts, which enter after performing a U-bend (Sawaya 1942; Gower & Wilkinson 2002). An extraordinary feature is the presence of Müllerian ducts, which become glandular during reproductive activity, and secrete a fluid containing lipids and sugars necessary for sperm motility (e.g. Wake 1981). The caudal phallodeum is more broadly built and the inner structure is very different. The ridges are arranged in a more complex pattern (running transversely). In adults it is often equipped with tuberosities or crests, which give the phallodeum a characteristic morphology and gives rise to an extraordinary variation in shape (Wiedersheim 1879; Tonutti 1931, 1933; Wake 1972; Exbrayat 1991; Gower & Wilkinson 2002, see also Fig 2), that is impotant for caecilian systematics (Müller et al. 2005). East-African scolecomorphid caecilians even have cartilaginous spicules (Wake 1998). In many species, pouchy dorsolateral appendixes – so called "blind sacs" – extend anterior to the phallodeum. During eversion, the luminal surface of the phallodeum represents the outer structure of the phallus, with the urodeum lying in-between (see Tonutti 1931; Gower & Wilkinson 2002, see also Fig. C right).

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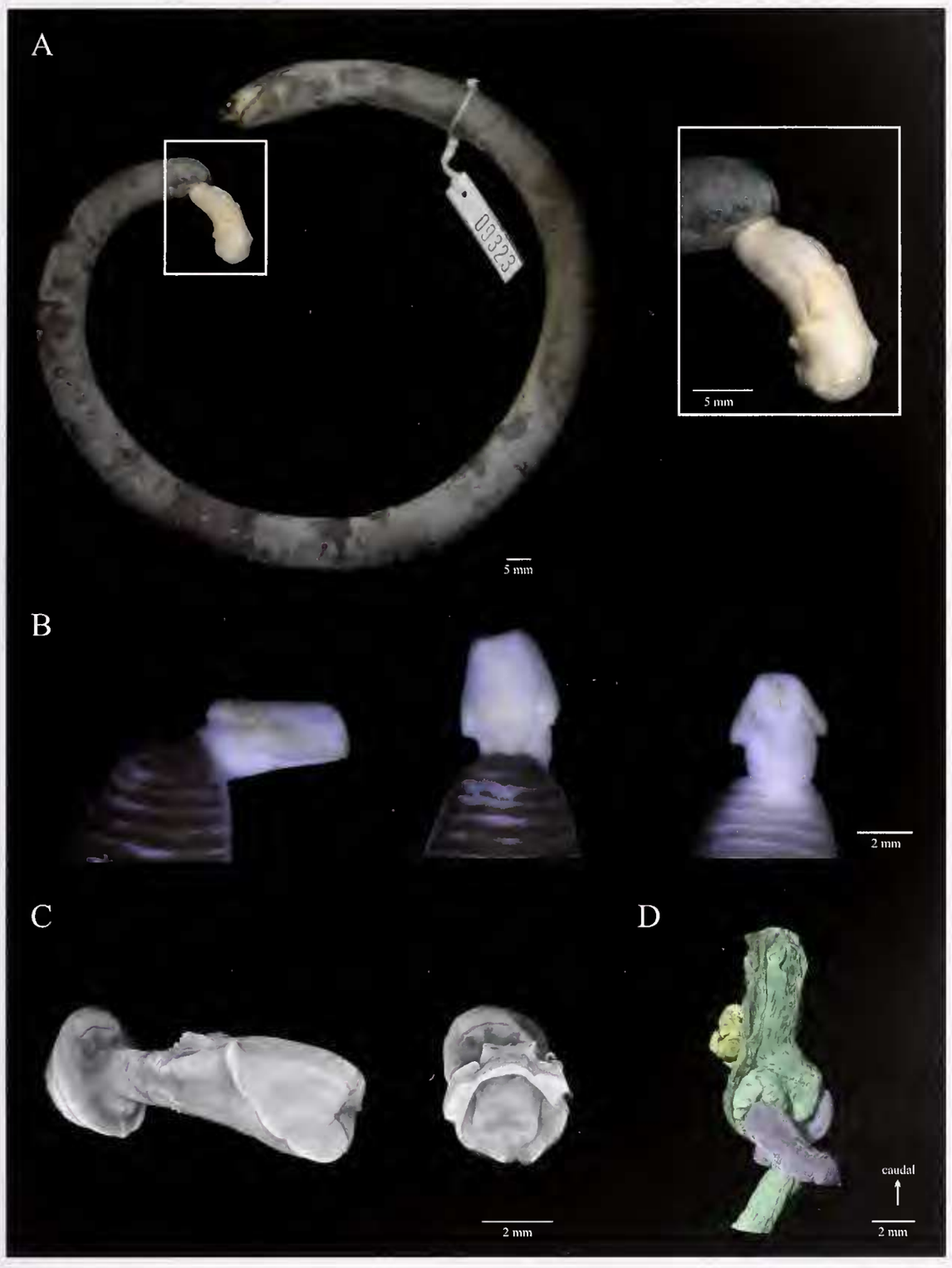

Fig. 2. Genital morphology of caecilian amphibians. (A) male *Chthonerpeton indistinctum* (Gymnophiona: Typhlonectidae) showing an everted phallus, MHNM 09323, right – detail. (B) *Geotrypetes seraphini* (Gymnophiona: Caeciliidae), lateral (left), dorsal (central) and ventral (right) view of the everted phallus, AK 01149. (C) *Typhlonectes natans* (Gymnophiona: Typhlonectidae), SRμCT-Scan of the everted phallus. Right – virtual clipping, frontal view. (D) SRμCT-Scan of female cloaca (*Ichthyophis* cf. *kohtaoensis*). Dorsolateral view, virtual cut of cloacal sheath, cranial part and blind sacs, green - cloaca, violet – oviducts, yellow – bladder. Abbreviations: MHNM = Museo Nacional de Historia Natural Montevideo Uruguay, AK = Alexander Kupfer collection.

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To retract the cloaca within the body after copulation, caecilians possess a specific muscle (musculus retractor cloacae), which is also found in some females (Wilkinson 1990).

The female cloaca of caecilians has received little attention (e.g. *Hypogeophis rostratus* Tonutti 1931; *Typhlonectes compressicauda*, Exbrayat 2006), the only dedicated morphological study was presented by Wake (1972), proposing a functional association between the specific male and female morphologies. The female cloaca is supposed to be non-eversible (Wilkinson 1990), therefore displaying a different morphology. Generally it is shorter than in males, and the urogenital ducts lack a copulatory loop (see Fig. 2D). There is also evidence for a bisection of the female cloaca (Exbrayat 1991; Kühnel et al. submitted). The cranial chamber is homologous to the male urodeum. The caudal chamber is marked by a different arrangement of longitudinal cloacal folds mostly lacking tuberosities, and therefore easily recognised. Nothing is at present known about how far the male phallus inserts into the female cloaca, and if special structures corresponding to the male ornamentation are present, helping in fixation during copulation.

Copulations in caecilians have rarely been observed. Data are available for two aquatic/semiaquatic species, the typhlonectids *Typhlonectes compressicauda* and *Chthonerpeton indistinctum*. Pairs of *C. indistinctum* copulated for betwen 30 minutes and 5 hours (Barrio 1969) and those of *T. compressicauda* for between 75 minutes and 3 hours (Murphy et al. 1977; Billo et al. 1985). Observations on copulations in terrestrial caecilians have, to the best of our knowledge, only been presented for the Indian ichthyophiid *Ichthyophis beddomei* (Bhatta 1999) and *Ichthyophis* cf. *kohtaoensis* (Kupfer et al. 2006a). Bhatta reports on a copulation lasting for about 40 or 45 minutes, an observation fitting well with the duration time of about 45 minutes that was observed in *Ichthyophis* cf. *kohtaoensis* (Kupfer et al. 2006a).

Caecilians show a remarkable diversity of reproductive modes associated with parental care (e.g. Wake 1977; Himstedt 1996; Wilkinson & Nussbaum 1998). Oviparous caecilians guarding egg clutches in terrestrial chambers (e.g. Sarasin & Sarasin 1887–1890) either have the presumed ancestral amphibian life cycle with aquatic larvae, or show direct development of juveniles with no aquatic larval stage (e.g. Brauer 1897). Females of viviparous species retain fertilised eggs. Embryogenesis is completed within the oviducts, and after hatching the foetuses feed mainly intrauterinely on the hypertrophied oviductal lining (e.g. Parker 1956; Welsch et al. 1977). After a long gestation period, the females give birth to fully metamorphosed, precocial young with the adult-type morphology (e.g. Billo et al. 1985; Exbrayat & Delsol 1985). Recently, a novel form of parental investment, maternal dermatotrophy, a.k.a. skin feeding, where altricial young feed externally on the mother´s hyperthrophied skin, has been described (Kupfer et al. 2006b; Wilkinson et al. 2008).

SUMMARY AND PERSPECTIVES

In addition to their remarkable diversity of reproductive modes, amphibians also show large variation in their reproductive morphology. Many morphological peculiarities are related to the evolution of internal fertilisation, and ultimately to viviparity. In relation to fertilisation and sperm transfer, different strategies have evolved within the three amphibian orders, ranging from cloacal apposition in anurans to a true copula via a highly complex male intromittent organ in caecilians. Amphibians offer a prime system for comparative studies of evolutionary reproductive biology. Research on the reproductive or genital morphology should include modern methodology, such as 3D reconstruction and soft tissue synchrotron radiation based X-ray microtomography (SRμCT, see Fig. 2 C–D). Because amphibian diversity is steadily increasing (although at the same time many species are declining or even going extinct) we envisage that many more unexpected reproductive strategies and morphologies remain to be discovered.

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